

**Amendments to the Specification:**

**Please insert the following new paragraph, at page 23, after line 30 and before line 31:**

BRIEF DESCRIPTION OF THE DRAWINGS

**Please insert the following new paragraph, at page 24, before line 1:**

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

**Please replace the paragraph at page 24, lines 1-10, with the following amended paragraph:**

**Figure 1:** A) FIG. 1A: Analysis by means of polyacrylamide in denaturing conditions (SDS-PAGE 12%) and coloring with Coomassie Blue of the result of the purification of the Fab fragment of the MNAC13 antibody (well 1: sample of MNAC13 antibody digested proteolytically with papaine; well 2: fraction bound to the DEAE Sephacell ionic exchange resin and eluted with NaCl 250 mM; well 3: molecular weights; well 4: Fab fragment of the purified and concentrated MNAC13 antibody)[[; B)]]].

FIG. 1B: Illustrates a typical crystal of the Fab fragment of the MNAC13 antibody. [[C)]]

FIG. 1C: High resolution diffraction spectrum obtained with a crystal of the Fab fragment of the MNAC13 antibody[[; D)]]].

FIG. 1D: Ramachandran chart of the torsion angles of the main chain of the heavy and light domains of the Fab fragment of the MNAC13 antibody.

**Please replace the paragraph at page 24, lines 11-19, with the following amended paragraph:**

**Figure 2:** A) FIG. 2A: Analysis by means of polyacrylamide in denaturing conditions (SDS-PAGE 12%) and coloring with Coomassie Blue of the result of the purification of the Fab fragment of the  $\alpha$ D11 antibody (well 1: sample of  $\alpha$ D11 antibody digested proteolytically with papaine; well 2: Fab fragment of the purified and concentrated  $\alpha$ D11 antibody; well 3: molecular weights)[[; B)]]].

FIG. 2B: A typical crystal of the Fab fragment of the  $\alpha$ D11 antibody. ~~[[C]]~~

FIG. 2C: High resolution diffraction spectrum obtained with a crystal of the Fab fragment of the  $\alpha$ D11 antibody~~[[; D]]~~ .

FIG. 2D: Ramachandran chart of the torsion angles of the main chain of the heavy and light domains of the Fab fragment of the  $\alpha$ D11 antibody.

**Please replace the paragraph at page 24, line 20 through page 25, line 2, with the following amended paragraphs:**

**Figure 3:** ~~A) B) C) D)~~ FIG. 3A, FIG. 3B, FIG. 3C, FIG. 3D: Distributions of the humanized or human origin antibodies (named using the PDB codes of their crystallographic structures) according to the three analyzed variables;~~E) F)~~.

FIG. 3E, FIG. 3F: Deviations of the humanized or human origin antibodies from the hypothetical optimal value of MNAC13 (calculated both considering the degree of overall identity and of homology – **in blue** – and framework level – **in magenta** –). ~~[[G]]~~

FIG. 3G: Structural alignment with the Fv fragment of MNAC13 of the respective regions of the humanized or human origin antibodies, selected according to the degree of identity and homology with the murine antibodies and to the degree of resolution of available structural data;~~H) I)~~.

FIG. 3H, FIG. 3I: Structural alignment with the Fv fragment of MNAC13 (shown in cyan) of the respective region of the selected humanized antibody 1AD0 (shown in red) in ~~[[H)];~~ FIG. 3H; of the model of the antibodies resulting after CDR grafting (shown in yellow at the framework level, in white at the CDR level) in ~~[[I)];~~ FIG. 3I. ~~[[L]]~~

FIG. 3J: Model of the Fv fragment of the MNAC13 humanized antibody obtained as a result of the identification of putative retro-mutations in the chosen framework (human origin residues are shown in green and murine origin residues are shown in magenta).

**Please replace the paragraph at page 25, lines 3-17, with the following amended paragraphs:**

**Figure 4:** ~~A) B) C) D)~~ FIG. 4A, FIG. 4B, FIG. 4C, FIG. 4D: Distributions of the humanized or

human origin antibodies (named using the PDB codes of their crystallographic structures) according to the three analyzed variables; E) F).

FIG. 4E, FIG. 4F: Deviations of the humanized or human origin antibodies from the hypothetical optimal value of  $\alpha$ D11 (calculated both considering the degree of overall identity and of homology – **in blue** – and framework level – **in magenta** –). [[G)]]

FIG. 4G: Structural alignment with the Fv fragment of  $\alpha$ D11 of the respective regions of the humanized or human origin antibodies, selected according to the degree of identity and homology with the murine antibodies and to the degree of resolution of available structural data; H) I).

FIG. 4H, FIG. 4I: Structural alignment with the Fv fragment of  $\alpha$ D11 (shown **in cyan**) of the respective region of the selected humanized antibody 1JPS (shown **in red**) in [[H);]] FIG. 4H of the model of the antibodies resulting after CDR grafting (shown **in yellow** at the framework level, **in white** at the CDR level) in [[I);]] FIG. 4I. [[L)]]

FIG. 4J: Model of the Fv fragment of the  $\alpha$ D11 humanized antibody obtained as a result of the identification of putative retro-mutations in the chosen framework (human origin residues are shown in cyan and murine origin residues are shown in purple).

**Please replace the paragraph at page 25, lines 18-24, with the following amended paragraph:**

**Figure 5:** FIG. 5: Alignment of the primary structures of the variable regions of the heavy chain (A) and of the light chain (B) respectively of MNAC13 (SEQ ID No. 22, SEQ ID No. 24), of the humanized antibody selected for humanization (1AD0; SEQ ID No. 39, SEQ ID No. 40), of the humanized form of MNAC13 after CDR grafting on the framework of 1AD0 and of the described retro-mutations and mutations (Hum MNAC13: SEQ ID No. 37, SEQ ID No. 38). CDRs are highlighted in the sequence of the humanized form of the two chains of MNAC13 by underlined character.

**Please replace the paragraph at page 25, lines 25-31, with the following amended paragraph:**

**Figure 6:** FIG. 6: Alignment of the primary structures of the variable regions of the heavy chain

(A) and of the light chain (B) respectively of D11 (SEQ ID No. 2, SEQ ID No. 4), of the humanized antibody selected for humanization (1JPS; SEQ ID No. 19, SEQ ID No. 20), of the humanized form of  $\alpha$ D11 after CDR grafting on the framework of 1AD0 and of the described retro-mutations and mutations ( $\alpha$ D11; SEQ ID No. 17, SEQ ID No. 18). CDRs are highlighted in the sequence of the humanized form of the two chains of  $\alpha$ D11 by underlined character.

**Please replace the paragraph at page 26, lines 1-13, with the following amended paragraph:**

**Figure 7:** FIG. 7: A) is the nucleotide sequence of the cDNA of the variable region of the light chain of the murine form of MNAC13 (SEQ ID No. 23); B) is the nucleotide sequence of the cDNA of the variable region of the heavy chain of the murine form of MNAC 13 (SEQ ID No. 21); C) and E) are the sequence sequences of the oligonucleotides drawn to obtain the humanized form of the variable region of the light chain of MNAC13 (SEQ ID No. 38): L1S: SEQ ID No. 31; L2AS: SEQ ID No. 32; L3S: SEQ ID No. 33; L4AS: SEQ ID No. 34; L5S: SEQ ID No. 35; L6AS: SEQ ID No. 36, by means of the overlap-assembly PCR technique, shown together with the corresponding translation into amino acid sequence; D and F) are the sequence sequences of the oligonucleotides drawn to obtain the humanized form of the variable region of the heavy chain of MNAC13 (SEQ ID No. 37): H1S: SEQ ID No. 25; H2AS: SEQ ID No. 26; H3S: SEQ ID No. 27; H4AS: SEQ ID No. 28; H5S: SEQ ID No. 29; H6AS: SEQ ID No. 30, by means of the overlap-assembly PCR technique, shown together with the corresponding translation into amino acid sequence.

**Please replace the paragraph at page 26, lines 14-21, with the following amended paragraph:**

**Figure 8:** FIG. 8: A) is the nucleotide sequence of the cDNA of the variable region of the light chain of the rat form of  $\alpha$ D11 (SEQ ID No. 3); B) is the nucleotide sequence of the cDNA of the variable region of the heavy chain of the murine form of  $\alpha$ D11 (SEQ ID No.1); C) and E) are the sequence sequences of the oligonucleotides drawn to obtain the humanized form of the variable region of the light chain of  $\alpha$ D11 (SEQ ID No. 18): L1S: SEQ ID No. 11; L2AS: SEQ ID No. 12; L3S: SEQ ID No. 13; L4AS: SEQ ID No. 14; L5S: SEQ ID No. 15; L6AS: SEQ ID No. 16,

by means of the overlap-assembly PCR technique, shown together with the corresponding translation into amino acid sequence; D and F) are the sequence sequences of the oligonucleotides drawn to obtain the humanized form of the variable region of the heavy chain of D11 (SEQ ID No. 17): H1S: SEQ ID No. 5; H2AS: SEQ ID No. 6; H3S: SEQ ID No. 7; H4AS: SEQ ID No. 8; H5S: SEQ ID No. 9; H6AS: SEQ ID No. 10, by means of the overlap-assembly PCR technique, shown together with the corresponding translation into amino acid sequence.

**Please replace the paragraph at page 26, line 28, through page 27, line 2, with the following amended paragraphs:**

**Figure 9:** FIG. 9A, FIG. 9B, FIG. 9C and FIG. 9D are Maps maps of the plasmids used to clone the sequences of the humanized variable regions of both antibodies obtained by overlap-assembly PCR. A) of

FIG. 9A: This is a map of the *pVLexpress* plasmid for the variable domain of the light chain[[,B]] .

FIG. 9B: This a map of the *pVHexpress* plasmid for the variable domain of the heavy chain[[, C)]].

FIG. 9C: This is a map of the plasmid resulting from cloning in *pVLexpress* the variable region of the light chain of each humanized antibody[[, D)]].

FIG. 9D: This is a map of alternative constructs obtained as a result of cloning in *pVHexpress* the variable region of the heavy chain of each humanized antibody: 1) for the expression in intact immunoglobulin form IgG1, 2) for expression in Fab fragment form, 3) for expression in immunotoxin form.

**Please replace the paragraph at page 27, lines 3-8, with the following amended paragraph:**

**Figure 10:** FIG. 10A: Comparison of the binding activity of the MNAC13 antibody in chimeric form and in humanized form by means of ELISA assay, conducted immobilizing on plastic TrkA in immunoadhesin form: A) comparison between serial dilutions of supernatants of transfected COS cells, subsequently concentrated; B)

between serial dilutions of supernatants of transfected COS cells, subsequently concentrated, of the binding activity of the MNAC13 antibody in chimeric form, and in humanized form, by means of ELISA assay, conducted with TrkA immobilized on plastic in immunoadhesin form.

FIG. 10B: ~~comparison~~ Comparison between serial dilutions of supernatants of transfected COS cells purified by means of G sepharose protein. OD 450 verses concentration.

FIG. 10C: Comparison between serial dilutions of supernatants of transfected COS cells purified by means of G sepharose protein. OD 450 verses log of concentration.

FIG. 10D: Comparison between serial dilutions of supernatants of transfected COS cells purified by means of G sepharose protein. OD 450 verses dilutions.

**Please replace the paragraph at page 27, lines 9-10, with the following amended paragraph:**

**Figure 11** FIG. 11: Assay of the binding activity of the  $\alpha$ D11 antibody in humanized form by means of ELISA assay, conducted ~~immobilizing on plastic~~ NGF with NGF immobilized on plastic.

**Please replace the paragraph at page 34, line 29 through page 35, line 2, with the following amended paragraph:**

As shown in ~~Figures 3 and 4~~, Figures 3A through 3J, and Figures 4A through 4J, the distributions of the antibodies considered in the space of the three analyzed variables (respectively, value of RMS, percentages of atoms on which RMS was calculated and a similitude index between primary structures, i.e. percentage of overall identity [[ -A-,]] Figures 3A and 4A, of overall homology [[ -C-,]] Figures 3C and 4C, of identity at the framework level [[ -B-,]] Figures 3B and 4B, of homology at the framework level [[ -D-,]] Figures 3D and 4D, are mutually coherent and consistent for both cases considered.

**Please replace the paragraph at page 37, lines 31-33, with the following amended paragraph:**

The ability to recognize the respective ligands of the two humanized antibodies was verified by means of ELISA assay and compared with respective chimeric forms. The

Application No.: 10/583,618

Docket No.: 128.1008

results are shown in ~~Figure 10 and 11~~ Figures 10A-10D and Figure 11.